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# PHYSICAL, CHEMICAL AND BIOLOGICAL PROPERTIES OF LUTEIN: A REVIEW

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## ABSTRACT

Lutein is along with β-carotene, one of the most widely distributed carotenoids in fruits and vegetables frequently consumed by different populations. Chemically lutein is the dihydroxy derivative of α-carotene presenting two hydroxyl group at the terminal rings of the molecules. Lutein plays an important role in Prevention of Hypoxia-Induced Cell Damage in the Eye, in Age-Related Macular Degeneration (AMD), in Diabetic Retinopathy, in Retinal Detachment, Lutein in the Lens and in the Uvea. Lutein has become known as the "eye vitamin" and its dietary intake is important in maintaining its concentration in human lens and retina. It also gives protection to the skin against UV light radiations and cancer. Lutein had an Inhibitory effect on Epstein-Barr virus activation in tumor activity to suppress the tumor promotion. Lutein showed the anti-tumor promoting activities in mouse two-stage skin carcinogenesis and decreased the number of aberrant crypt foci in rat colon. Lutein suppressed tumorigenesis in skin and colon in mice.

**KEY WORDS:** Lutein, eye protection, anticancer, antioxidant, Carotenoid.

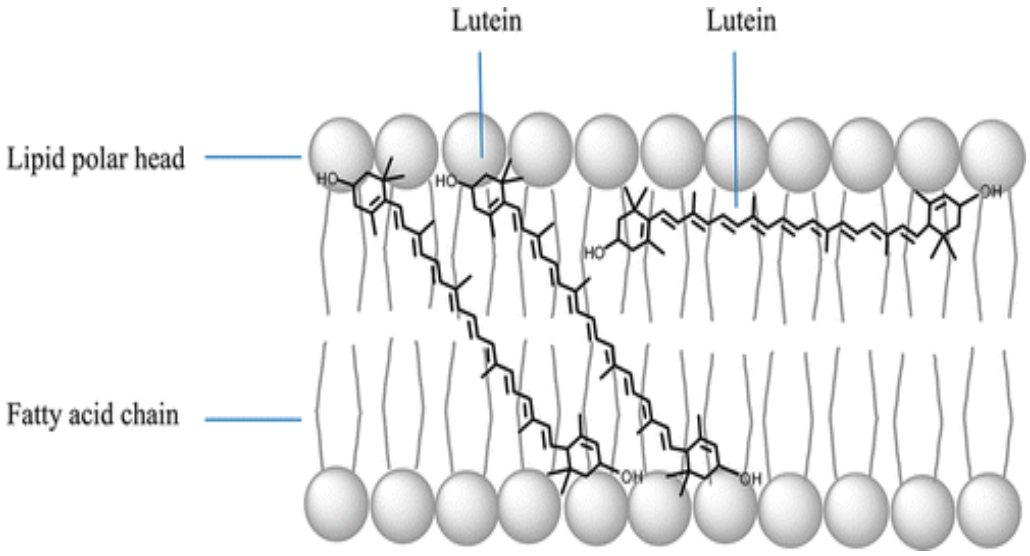
## INTRODUCTION

Lutein is a plant pigment that belongs to the well known group of carotenoids with the molecular formula $C_{40}H_{56}O_2$.. Man is not capable of synthesizing carotenoids de novo and thus their presence in human tissues is entirely of dietary origin although man is capable of modifying some of them to some extent. Lutein is along with β-carotene, one of the most widely distributed carotenoids in fruits and vegetables frequently consumed by different

populations. Lutein: is a *Carotene*-3,3'-diol with the molecular weight 568.871. Its melting point is 183-185$^0$ C ([1,2,3,4]).

**Lutein absorbance and distribution ([5])**

Chemically lutein is the dihydroxy derivative of α- carotene presenting two hydroxyl group at the terminal rings of the molecules. In food, lutein can be found either in its free form, bound to proteins or esterified as a monoester or diester. After being released from the food matrix, it is incorporated into micells to be absorbed by passive by enterocytes and along with other carotenoids and other fat molecules dietary compounds is incorporated into nascent chylomicrons for transport to the liver. In blood, lutein is transported by lipoprotein with an even distribution among different classes.




Because of its polarity, it is assumed to be located at the lipoprotein surface and thus is more readily transferred among the different classess of lipoprotein even during post prandial metabolism Distribution of lutein among the tissues is similar to that of other carotenoids and is determined atleast partly by LDL receptors density lutein is selectively accumulated in different parts of the human eye. Lutein is especially abdundant at the centre of the retina (macula) thus usually referred as mascular pigment

**Nutritional relevance of lutein ([6,7])**

Traditionally the nutritional importance of carotenoids in man has rested on their provitamin A activity. However because of the presence of hydroxylated terminal rings in its structure, lutein does not fit the structural requirement for its activity and thus shows no activity in man.

**Relationship between lutein intake and their clinical end point. ([8,,9,10])**

It is assumed that increased exposure to that nutrient usually measured by intake or serum levels is capable of enhancing a relevant biochemical indicators and or function which in turn considered to be casually related to the modification of a disease process that is casually related to health outcome. . Traditionally lutein intake or nutritional status assessment in human subject has been routinely performed by dietary and biochemical methods, both of which have advantage and limitation. Because of the selective deposition of lutein in retina, its relevance to other tissue, intermediate biomarkers and non-0cular clinical conditions remins to be established.

**Occurrence of Lutein ( [11,12,13,14,15] )**

Lutein is found in egg yolk and in many plants and vegetables, including red peppers, mustard, broccoli, zucchini, corn, garden peas, spinach, leek, collard greens and kale. Lutein is responsible for the coloring of many fruits and vegetables. Lutein is an antioxidant which is believed to be an essential nutrient for normal vision.. Sunflower is a special source for isolating lutein.

**Isolation of Lutein from *Helianthus annuus* L. petal extract**

Lutein was isolated by suspending 5g of petal powder in 100ml of hexane. The resultant suspension was homogenized for 15min, filtered through Whatmann No.1 filter paper and the volume was measured. The residue (pellet) of extract was weighed after drying to constant weight to calculate the solubility of the petal powder. The solubility was found to be 30%. The filtrate was evaporated at $55^{O}C$ and concentrated to half of its volume obtained originally. The above prepared hexane extract, 10ml of was added to 10% KOH in 20ml ethanol. The solution was evaporated at $68^{O}C$ for 15min. The resultant solution was washed with 30ml of 1:1 mixture of water:ethanol, centrifuged the mixture at 8000rpm for 10min at $10^{O}C$. The resultant residue was re-constituted in small volume of 1:1 mixture of water :ethanol.

**Spectral analysis of Lutein isolated from *Helianthus annuus* L. ([16])**

Spectral analysis of Lutein standard (10µg/20µl) and Lutein fraction isolated from *Helianthus annuus* L. petal methanol extract (10µl) was scanned between the wavelength region of 400–500nm. The appearance of signature wavelength for Lutein at 421, 446 and 472nm was recorded in Hitachi 2900UV double beam spectrophotometer.

**Spectral Data for Lutein ([17,18,19,20,21])**

**1. UV:** λ max (nm): : dioxane 429 (1680), 453 (2515), 482 (2259) methanol 330, 422, 443, 470

**2. NMR: & 1H-NMR** 1H-NMR (270 MHz, CDCl3): 0.849, 0.998 (6H, s, 1'-gem-Me), 1.074 (6H, s, 1-gem-Me), 1.37 (1H, dd, J 13, 7, 2'ax-H), 1.48 (1H, t, J 12, 2ax-H), 1.626 (3H, s, 5'-Me), 1.739 (3H, s, 5-Me), 1.84 (1H, dd, J 13, 6, 2'eq-H), 1.912 (3H, s, 9'-Me), 1.970 (9H, s, 9-, 13-, 13'- Me), 2.04 (1H, dd, J 17, 10, 4ax-H), ca. 2.33-2.45 (2H, m, 6'-, 4eq-H), ca. 4.0 (1H, m, 3-H), 4.25 (1H, 3'-H), 5.43 (1H, dd, J 15.5, 10, 7'-H), 5.55 (1H, s, 4'-H), ca. 6.12 (2H, s, 7-, 8-H), ca. 6.15 (3H, m, 8'-, 10-, 10'-H), ca. 6.26 (2H, m, 14-, 14'- H), 6.36 (2H, d, J 15, 12-, 12'-H), ca. 6.55-6.71 (4H, m, 11-, 11'-, 15-, 15'-H)

**3. 13C-NMR &** (CDCl3): 13C-NMR (CDCl3): 37.1 (C1), 48.4 (C2), 65.1 (C3), 42.5 (C4), 126.2 (C5), 137.6 (C6), 125.6 (C7), 138.5 (C8), 135.6 (C9), 131.3 (C10), 124.9 (C11), 137.6 (C12), 136.5 (C13), 132.6 (C14), 130.0 (C15), 28.7, 30.2 (1- gem-Me), 21.6 (5-Me), 12.7 (9-Me), 12.7 (13-Me), 34.0 (C1'), 44.7 (C2'), 65.9 (C3'), 125.6 (C4'), 137.8 (C5'), 55.0 (C6'), 128.6 (C7'), 137.8 (C8'), 135.0 (C9'), 130.8 (C10'), 124.5 (C11'), 137.6 (C12'), 136.5 (C13'), 132.6 (C14'), 130.0 (C15'), 24.3, 29.5 (1'-gem-Me), 22.8 (5'-Me), 13.2 (9'- Me), 12.7 (13'-Me)

**Biological properties of Lutein ([22,23]).**

Lutein improves heart health, protects our skin against UV damage, reduces diabetes induced oxidative stress, and possesses anti-inflammatory and anti-cancer properties. The central part of the retina, called the macula, contains macular pigments in which Lutein is concentrated. The yellow colored pigments protect the retina from damage of the photo-oxidative affect of high-energy radiations. Lutein offers eye protection by lowering the risk of age related vision loss, which causes gradual loss of central vision. Age related vision loss or age related macular degeneration is caused by steady damage of the retina. There are reports which determine the effect of food supplements or eating foods rich in antioxidants that can protect against age-related macular degeneration, a disease in which the central portion of the retina deteriorates so that only peripheral vision remains.

**Role of Lutein in Prevention of Hypoxia-Induced Cell Damage in the Eye**

Free radicals which have one or more unpaired electrons in an atom or groups of atoms, come in many different forms. The most common type of free radical in biological system is the

reactive oxygen species (ROS) which are generated in the retina because of oxygen ([24,15]) consumption as well as high energy photon light conversion into electrochemical signaling. The accumulation of oxygen radicals and lipid peroxidation, resulting from increased retinal oxygen utilization, has been postulated as a mechanism for photoreceptor apoptosis via caspase 9 activation.([26,27,28]). One of the major protective roles lutein has in the retina is to serve as an oxygen free radical scavenger during oxidative stress conditions. The ability of lutein to provide effective removal of free radicals, such as singlet oxygen particles, is primarily governed by the chemical structure of two hydroxyl groups acting as strong sinks for reactive oxygen species The effective dose ($ED_{50}$) value of lutein as a free radical scavenger is 0.7 μM ([29,30]). In addition, lutein selectively absorbs blue light, due to its peak absorption spectrum of 446 nm. Blue light produces more light induced damage (100-fold) than orange light, depending on the exposure time. As a consequence of its filtering capabilities, lutein is effective in preventing photoreceptor damage produced by blue light ( [31]).

In retinal pigment epithelial cells, fed native or UV-irradiated photoreceptor outer segments and cultured in 40% oxygen, lutein significantly reduced lipofuscin formation ([32,33])˙. In RGC-5 rat ganglion cell lines, lutein treatment prevented cellular death following oxidative damage via $H_2O_2$. Similar results were obtained in immortalized Müller cells ([34,35])

**Role of Lutein in Age-Related Macular Degeneration (AMD)**

The presence of carotenoids in the macula capable of absorbing light of the blue range wavelength would indicate that they serve a protective function. Specifically lutein appears to play a specific role as a photo protective agent, effectively screening out the damaging blue light from causing excessive damage on the photoreceptors ([36])

AMD is a degenerative process of the macula and is the principle cause of blindness among people age 65 and older in Western countries. AMD can be classified into two categories: non-exudative (or dry) and exudative (or wet) AMD. The former is characterized by accumulation of soft drusen caused by photo-oxidative damage and de-pigmentation of the retinal pigment epithelium. The latter is characterized by neovascularization of the macula, and accumulation of scar tissue ([37])

AMD is a multifactorial disease. Among the important risk factors for AMD are age, genetic susceptibility, sunlight exposure, cigarette smoking, and poor nutritional status.

Combined with the fact that lutein and zeaxanthin are the only carotenoids found in the macula and comprise the macular pigment, this suggests that the observed protective effects of high fruit and vegetable intake may be due primarily to lutein and zeaxanthin intake.([38]).

**Lutein Role in Diabetic Retinopathy**

In a clinical study that has been carried out in the peoples with diabetic retinopathy, showed that the serum concentration of lutein and zeaxanthin was significantly lower in the diabetic patients than the normal peoples. Results of the clinical study suggested that lutein and zeaxanthin supplementation in patients led to improvement of visual acuity and decrease in foveal thickness ([39]) and also suggests that lutein and zeaxanthin supplementation might be potentially used as therapeutic agents in treating non-proliferative diabetic retinopathy.

**Lutein in Retinal Detachment**

A clinical study was carried in patients with retinal detachment. In that study it was found the presence of high levels of lutein and retinol and very little β-carotene in the sub retinal fluid**.** Lutein was the major carotenoid peak in sub retinal fluid (41.4 ± 14.1 ng/mL). high proportion of lutein and very low amount of β-carotene in the sub retinal fluid support the occurrence of a highly selective transport mechanism of lutein from the blood to the retina ([40])

**Lutein in the Lens**

Lutein and zeaxanthin are the only carotenoids present in the crystalline lens ([41,42]). Cataract is the opacification of the crystalline lens and is caused by precipitation of lens proteins. The development of cataract is facilitated by oxidative damage and often results in impaired vision or blindness. It was suggested that lutein and zea xanthin may retard aging of the lens ([43])

**Lutein and the Uvea**

Uveitis, a common ophthalmic disorder, is responsible for approximately 10% of blindness ([44,45]). It may be caused by autoimmune disorders, infections or exposure to toxins. Reactive oxygen species (ROS) play an important role in mediating the inflammatory signals induced by lipopolysaccharides (LPS). It is suggested that natural antioxidants exert protective effects on the LPS-induced uveitis ([46] ). Oral administration of lutein (125 and 500 mg/kg/day for five days) reduced the nitric oxide level in eye tissues in an animal model ([47]) The same study showed that lutein decreased the malondialdehyde content, increased

the oxygen radical absorbance capacity level, glutathione, the vitamin C contents and total superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities and further increased expressions of copper-zinc SOD, manganese SOD and GPxm RNA. Hence the antioxidant properties of lutein contributed to the protection against LPS-induced uveitis, partially through interfering with the inflammatory process.

Multiple animal studies on the neuroprotective effects of lutein against retinal neural damage caused by inflammation in endotoxin-induced uveitis (EIU) ([48]) have shown that the lutein has a dose-dependent anti-inflammatory effect on EIU. The possible mechanism for this effect of lutein may depend on its ability to inhibit the activation of NF-κB and the subsequent inhibition of pro-inflammatory mediators.

**Protection of skin against UV light radiations by lutein ( [49,50,51,52,53]).**

High intake of carotenoids –rich food is associated with reduced incidence of many forms of cancer and this effect is due to antioxidant properties of compounds. Carotenoids are present in epidermis and dermis are believed to play an important part in the skin's antioxidant defense system.. lutein protects the skin from local UV B radiations induced immune suppression but lutein has no effect in the systemic model of UVB radiations induced immunosuppressant. Lutein protects from sun burn reactions. Lutein protects from local UVB radiations induced immune suppression. Dietary lutein increases murine skin content level. Dietary lutein reduces ROS generation in Murine skin.

**Protection of lutein against cancer**

Time course studies with a constant concentration of 2.0 µg/ml of lutein showed significant inhibition of malignant AT3 cells. But increased concentration of lutein 10 µg/ml did not showed significant effect on cell growth. Lutein was found to have inhibitory effect on the malignant cell lines AT3.([54])

Lutein had an Inhibitory effect on Epstein-Barr virus activation (anti-tumor promotion) ([55]), Inhibitory effect on colonic aberrant crypt foci formation (anti-tumor promotion) **(**[56]). Lutein showed the anti-tumor promoting activities in mouse two-stage skin carcinogenesis and decreased the number of aberrant crypt foci in rat colon ([57]) Lutein decreased the number of aberrant crypt foci in colons in mice**..** Lutein and suppressed tumorigenesis in skin and colon in mice **(**[58,59])

**Lutein Supplementation**

Since lutein is completely insoluble in water, its incorporation into carrier systems has been studied to optimize its nutritional delivery method. Traditionally, lutein is dissolved in an organic solvent and nano-assemblies are obtained by emulsion–solvent evaporation, associating lutein with the amphiphilic cyclodextrin C4:7 at 1:6 molar ratio in aqueous medium. The nano-assemblies allow increased carotenoid solubility in water compared to carotenoid by itself.

It has been suggested that 6 mg of lutein per day, either through diet or using supplements is likely effective in reducing the risk of cataracts and AMD. Although the optimal dose for lutein supplementation has not been established yet, the most common dose in commercial products is 10 mg/day. Lutein is found in many natural products including broccoli, spinach, kale, corn, orange pepper, kiwi fruit, grapes, orange juice, zucchini, and squash. There is 44 mg of lutein per cup of cooked kale, 26 mg/cup of cooked spinach, and 3 mg/cup of broccoli. Toxicity of dietary intake of lutein (supplemental 35 mg per day) has been studied in rats and results show no serious adverse effects.

**DISCUSSION AND CONCLUSIONS**

Lutein and lycopene are naturally occurring substances found in many plants. Carotenoids in general have undergone a number of research studies as to their possible benefits against diseases, among other health issues. Lycopene is considered as antioxidant and anticancer agents while lutein showed marked effects on ocular conditions specially cataract. Lutein is particularly being studied for its effects on the human eyes. Lutein and lycopene are both found in numerous foods.

There are several epidemiological studies that link lutein supplementation with decreased risk of AMD. Lutein supplementation has also been positively linked to increased macular pigment density and improved multifocal electroretingram responses. Building on the findings of the original Age-Related Eye Disease Study, the AREDS2 study is further investigating whether supplementation with lutein and zeaxanthin, in addition to original AREDS formula, would add additional benefit in improving AMD outcomes. Lutein has a significant role in protecting against AMD, most likely through its absorption of the harmful blue light, as well as its inherent antioxidant properties. The role of lutein in protecting against diabetic retinopathy is not as well established as it is the case for AMD. Serum levels of lutein has been shown to be lower in NPDR patients and lutein supplementation has been

shown to improve NPDR. The antioxidant properties of lutein would likely explain its protective function in diabetic retinopathy. Further epidemiologic studies are needed to establish a stronger link between lutein and improvement of diabetic retinopathy.

Several lines of evidence have also suggested a protective role for lutein in the development of nuclear sclerosis cataracts. Considering that oxidative damage of lens proteins plays a major role in development of cataracts, anti-oxidative functions of lutein explain its likely role in slowing the formation of cataracts.

There are weaker lines of evidence to suggest protective roles for lutein against uveitis, and its possible role in the pathogenesis of retinal detachment. Further studies are needed to clarify whether lutein has a role in these ocular diseases.

In conclusion, the antioxidant, anti-inflammatory and blue light-absorptive properties of lutein provide its many protective roles in various ocular diseases especially AMD and cataracts. Lutein has become known as the "eye vitamin" and its dietary intake is important in maintaining its concentration in human lens and retina.

**REFERENCE**

1. Widmer E, Soukup M, Zell R, Broger E, Wagner HP and Imfeld M. Technical Procedures for the Syntheses of Carotenoids and Related Compounds from 6-Oxo-isophorone: Syntheses of (3R,3'R)-Zeaxanthin Part I, Helv. Chim. Acta.1990; 73: 861 - 867.
2. Landrum JT and Bone RA. Lutein, zeaxanthin, and the macular pigment. Arch Biochem Biophys. 2001; 385: 28-40.
3. Johnson EJ. The role of carotenoids in human health. Nutr Clin Care 2002;5::56-65.
4. Olaf Sommerburg, Jan E E Keunen, Alan C Bird, Frederik J G M van Kuijk, Fruits and vegetables that are sources for lutein and zeaxanthin: the macular pigment in human eyes, Br J Ophthalmol 1998: 82: 907–910.
5. Khachik, F., Bernstein, PS. And Garland, D L Identification of lutein and zeaxanthin oxidation products in human and monkey retinas. Investig. Ophthalmol. Vis. Sci. 1997; 38: 1802–1811.
6. Taylor A, Jacques PF, Chylack LT. Long-term intake of vitamins and carotenoids and odds of early age-related cortical and posterior subcapsular lens opacities. Am J Clin Nutr 2002;75: 540–549.

7. Thurnham DI, Northrop-Clewes CA and Chopra M. Biomarkers of vegetable and fruit intakes. Am J Clin Nutr. 1998; 68:756–757.
8. Toniolo P, Van Poppel AL, Akhmedkhanov A. Serum carotenoids and breast cancer. Am J Epidemiol. 2001; 153: 1142–1147.
9. Van den Berg H, Faulks R, Granado F. The potential for the improvement of carotenoid levels in foods and the likely systemic effects. J Sci Food Agric. 2000; 80: 880–913.
10. Garcı´a R, Gonza´lez CA, Agudo A and Riboli E (1999) High intake of specific carotenoids and flavonoids does not reduce the risk of bladder cancer. Nutr Cancer. 1999; 35: 212–214.
11. Weisburger JH. Evaluation of the evidence on the role of tomato products in disease prevention. Proc Soc Exp Biol Med. 1998; 218:140–143.
12. Zofia Olempska-Beer. Lycopene from B. Trispora , chemical and technical assessment (CTA). Chemical and Technical. 2006; 3(6): 78-84.
13. . Sies H, Stahl W. Lycopene: antioxidant and biological effects and its bioavailability in the human. Proc Soc Exp Biol Med. 1998; 218:121–124.
14. Rao AV, Agarwal S. Bioavailability and in vivo antioxidant properties of lycopene from tomato products and their possible role in the prevention of cancer. Nutr Cancer. 1998; 31:199–203.
15. Hoppe PP, Kramer K, Van Den Berg H. Synthetic and tomato-based lycopene have identical bioavailability in humans. Eur J Nutr. 2003; 42:272-278.
16. Sujatha R, Srinivas L. Biological properties of helianthus annuus Toxicol In vitro 1995; 9: 231–236.
17. Takaichi S and Shimada K. Characterization of carotenoids in photosynthetic bacteria, Methods Enzymol. 1992; 213: 374 -385.
18. Vecchi M, Englert G, and Mayer HM. Chromato graphische Trennung und Identifizierung diastereomerer Carotinoide mitgrossem räumlichem Abstand der chiralen Zentren, Helv. Chim. Acta, 1982; 65:1050 - 1058.
19. Sasa T, Takaichi S, Hatakeyama N, and Watanabe MM. A novel carotenoid ester, loroxanthin dodecenoate, from Pyramimonas parkeae, Plant Cell Physiol. 1992; 33: :921 - 925.
20. Moss GP. Carbon-13 NMR spectra of carotenoids, Pure Appl. Chem.. 2007; 20(47): 97 - 102.

21. Mayer H, and Rüttimann A. Synthese von optisch aktiven, natürlichen Carotinoiden und strukturell verwandeten Naturprodukten IV. Synthese von (3R,2'R,6'R)-Lutein, Helv. Chim. Acta, 1980; 63: 1451 -1455.
22. Leung, IY. Macular pigment: New clinical methods of detection and the role of carotenoids in age-related macular degeneration. Optometry. 2008, 79, 266–272.
23. Bhosale, P. Serban, B, Zhao, da Y, Bernstein, PS. Identification and metabolic transformations of carotenoids in ocular tissues of the Japanese quail Coturnix japonica. Biochemistry 2007; 46: 9050–9057.
24. Bhosale, P, Bernstein, PS. Quantitative measurement of 3'-oxolutein from human retina by normal-phase high-performance liquid chromatography coupled to atmospheric pressure chemical ionization mass spectrometry. Anal. Biochem. 2005; 345: 296–301.
25. Khachik, F, Carvalho, L, Bernstein, PS, Muir, GJ, Zhao, DY, Katz, NB. Chemistry, distribution, and metabolism of tomato carotenoids and their impact on human health. Exp. Biol. Med. (Maywood) 2002; 227: 845–851.
26. Siems WG, Sommerburg O, Van Kuijk, FJ. Lycopene and β-carotene decompose more rapidly than lutein and zeaxanthin upon exposure to various pro-oxidants *in vitro*. Biofactors. 1999; 10: 105–113.
27. Panfoli I, Calzia D, Ravera S, Morelli AM, Traverso CE. Extra-mitochondrial aerobic metabolism in retinal rod outer segments: New perspectives in retinopathies. Med. Hypotheses. 2012; 78: 423–427.
28. Bone, RA, Landrum JT. Macular pigment in Henle fiber membranes: A model for Haidinger's brushes. Vis. Res. 1984; 24: 103–108.
29. Landrum J, Bone R, Mendez V, Valenciaga A, Babino D. Comparison of dietary supplementation with lutein diacetate and lutein: A pilot study of the effects on serum and macular pigment. Acta Biochim. Pol. 2012; 59: 167–169.
30. Qin L, Bartlett H, Griffiths HR, Eperjesi F, Armstrong RA, Gherghel D. Macular pigment optical density is related to blood glutathione levels in healthy individuals. Investig. Ophthalmol. Vis. Sci. 2011; 52: 5029–5033.
31. Nilsson SE, Sundelin SP, Wihlmark U, Brunk UT. Aging of cultured retinal pigment epithelial cells: oxidative reactions, lipofuscin formation and blue light damage. Doc. Ophthalmol. 2003; 106: 13–16.
32. Lornejad-Schafer MR, Lambert C, Breithaupt DE, Biesalski HK, Frank J. Solubility, uptake and biocompatibility of lutein and zeaxanthin delivered to cultured human retinal pigment epithelial cells in tween40 micelles. Eur. J. Nutr. 2007; 46: 79–86.

33. Kalariya NM, Ramana KV, Srivastava SK, van Kuijk FJ. Genotoxic effects of carotenoid breakdown products in human retinal pigment epithelial cells. Curr. Eye Res. 2009; 34: 737–747.
34. Sasaki M, Ozawa Y, Kurihara T, Noda K, Imamura Y, Kobayashi S, Ishida S, Tsubota K. Neuroprotective effect of an antioxidant, lutein, during retinal inflammation. Investig. Ophthalmol. Vis. Sci. 2009; 50: 1433–1439.
35. Li SY, Lo AC. Lutein protects RGC-5 cells against hypoxia and oxidative stress. Int. J. Mol. Sci. 2010; 11: 2109–2117.
36. Handelman GJ, Dratz EA, Reay CC, van Kuijk JG: Carotenoids in the human macula and whole retina. Inves. Ophthalmol Vis Sci.1988; 29: 850-855.
37. Klein R, Rowland MR, Harris MI: Racial/ethnic differences in age related maculopathy. Third National Health and Nutrition Examination Survey. Ophthalmology 1995; 102: 371-378.
38. Holz FG, Piguet B, Minassian DC, Bird AC, Weale RA: Decreasing stromal iris pigmentation as a risk factor for age-related macular degeneration. Am J Ophthalmol 1994; 117: 19-23.
39. Hu BJ, Hu YN, Lin S, Ma WJ, Li XR. Application of Lutein and Zeaxanthin in non proliferative diabetic retinopathy. Int. J. Ophthalmol. 2011; 4: 303–306.
40. Chan C, Leung I, Lam KW, Tso MO. The occurrence of retinol and carotenoids in human sub-retinal fluid. Curr. Eye Res. 1998; 17: 890–895.
41. Gao S, Qin T, Liu Z, Caceres MA, Ronchi CF, Chen CY, Yeum KJ, Taylor, A, Blumberg JB, Liu Y. Lutein and zeaxanthin supplementation reduces $H_2O_2$-induced oxidative damage in human lens epithelial cells. Mol. Vis. 2011; 17: 3180–3190.
42. Zhao B, He YY, Chignell CF, Yin JJ, Andley U, Roberts JE. Difference in phototoxicity of cyclodextrin complexed fullerene [(gamma-CyD)2/C60] and its aggregated derivatives toward human lens epithelial cells. Chem. Res. Toxicol. 2009; 22: 660–667.
43. Berendschot TT, Broekmans WM, Klopping-Ketelaars IA, Kardinaal AF van Poppel G, Van Norren D. . Lens aging in relation to nutritional determinants and possible risk factors for age-related cataract. Arch. Ophthalmol. 2002; 120: 1732–1737.
44. Suhler EB, Lloyd MJ, Choi D, Rosenbaum JT, Austin DF. Incidence and prevalence of uveitis in Veterans Affairs Medical Centers of the Pacific Northwest. Am. J. Ophthalmol. 2008; 146: 890–896.

45. Gritz DC, Wong IG. Incidence and prevalence of uveitis in Northern California; the Northern California Epidemiology of Uveitis Study. Ophthalmology 2004; 111: 491–500.
46. Yao N, Lan F, He RR, Kurihara H. Protective effects of bilberry (Vaccinium myrtillus L.) extract against endotoxin-induced uveitis in mice. J. Agric. Food Chem. 2010; 58: 4731–4736.
47. He RR, Tsoi B, Lan F, Yao N. Yao, X.S.; Kurihara, H. Antioxidant properties of lutein contribute to the protection against lipo polysaccharide-induced uveitis in mice. Chin. Med. 2011; 6: 38.
48. Jin XH, Ohgami K, Shiratori K, Suzuki Y, Hirano T, Koyama Y, Yoshida K Ilieva I, Iseki K, Ohno S. . Inhibitory effects of lutein on endotoxin-induced uveitis in Lewis rats. Investig. Ophthalmol. Vis. Sci. 2006; 47: 2562–2568.
49. Black HS. Potential involvement of free radical reaction of UV light mediated cutaneous damage. Photochemistry and Photobiology. 1987; 46: 213-221.
50. Darr D, Fridovich I. Free radical in cutaneous biology. Journal of investigational dermatology. 1994; 102: 671-675.
51. Kripe ML, Immunological unresponsiveness induced by ultraviolet radiation. Immunology review.1984; 80:87-102.
52. Landrum JT and Bone RA. Lutein ,Zeaxanthin and the mascular pigment. Archieves of biochemistry and Biophysics.2001; 385: 28-40.
53. Mathew Rotu MM, Krinsky N. Carotenoids effect the development of UVB – induced skin cancer. Photochemistry and Photobiology. 1987; 46: 507-509.
54. Richard S, Gunasekera, Kiran Sewgobind, Smruthi Desai, Larry Dunn, Momerr S. Black, Wallace L. Mckeehan and Bhimmanagouda Patil. Lucopene and Lutein inhibit proliferation in rat prostrate carcinoma cells. Nutrition and cancer. 2007; 58 (2): 171-177.
55. Tsushima M, Maoka T, Katsuyama M, Kozuka M, Matsuno T, Tokuda, H, Nishino H, and Washima, A. Inhibitory effect of natural carotenoids on Epstein-Barr virus activation activity of a tumor promoter in Raji cells. A screening study for anti-tumor promoters, Biol Pharm Bull. 1995; 18: 227-233.
56. Narisawa T, Fukaura Y, Hasebe M, Ito M, Aizawa R, Murakoshi M, Uemura S, Khachik F, and Nishino H. Inhibitory effects of natural carotenoids, alpha-carotene, beta-carotene, lycopene and lutein, on colonic aberrant crypt foci formation in rats, Cancer Lett., 1996; 107: 137-142.
57. Nishino H. Cancer prevention by natural carotenoids. J Cell Biochem Suppl. 1997; 27: 86-91.

58. Kim JM, Araki S, Kim DJ, Park CB, Takasuka N, Baba-Toriyama H, Ota T, Nir Z, Khachik F, Shimidzu N. Chemo preventive effects of carotenoids and curcumins on mouse colon carcinogenesis after 1,2-dimethylhydrazine initiation, Carcinogenesis., 1998; 19: 81- 85 .
59. Nishino H, Tokuda H, Murakoshi M, Satomi Y, Masuda M, Onozuka M, Yamaguchi S, Takayasu J, Tsuruta J, Okuda M. Cancer prevention by natural carotenoids, Biofactors. 2000; 13: 89-94.
60. WebMD L: Lutein. Edited by WebMd Editorial Team, 2013. Available online: http://www.webmd.com/vitamins-supplements/ingredientmono-754 lutein.aspx? active Ingredient Id=754& activeIngredientName=lutein (accessed on 19 April 2014).
61. Harikumar KB, Nimita CV, Preethi KC, Kuttan R, Shankaranarayana ML, Deshpande. J. Toxicity profile of lutein and lutein ester isolated from marigold flowers (Tagetes erecta). Int. J. Toxicol. 2008; 27: 1–9.